

Figure1
Rat Nedd4

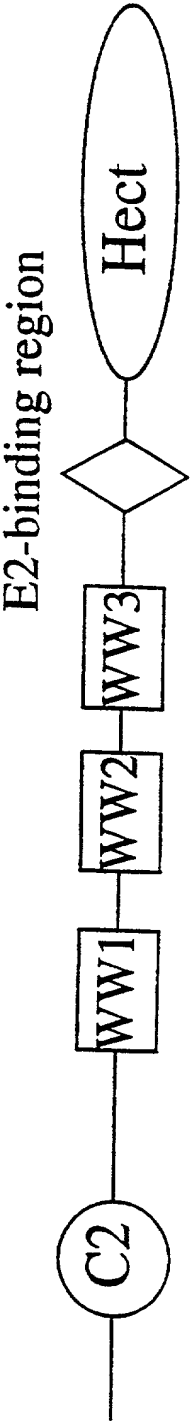
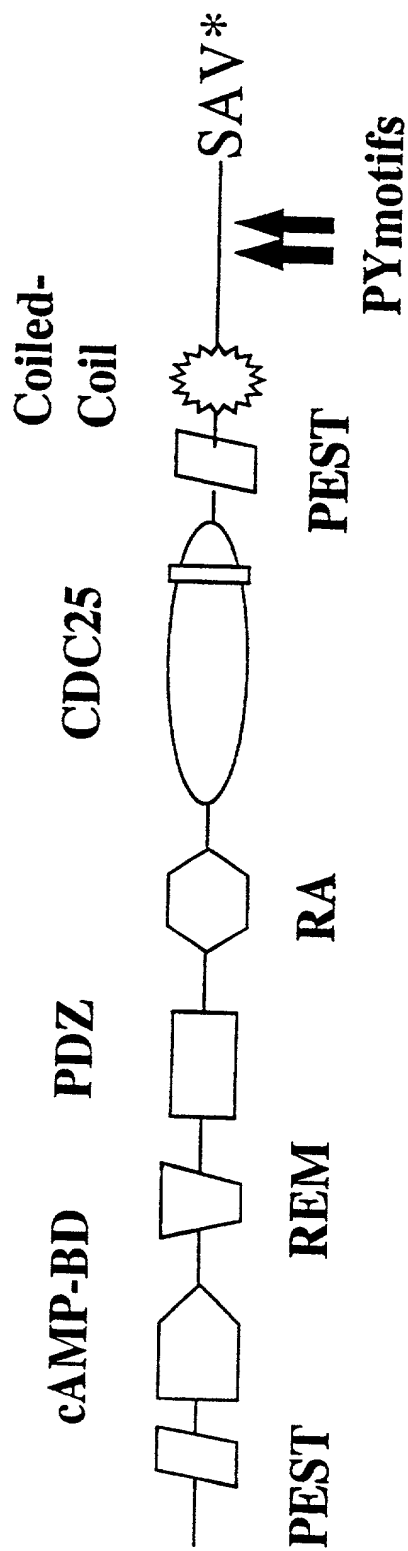


Figure 2: Clone 7.7 is the homolog of human clone KIAA0313

- Clone 7.7 exhibits 75% identity and 95% similarity of human clone KIAA0313.

Clone7.7 KIAA0313	GGKDVSAEAESSSMVPVTTEEAKVPMPAHIAVTPSTTKGLIARKEGRYREPPPTPPGYV GGKDVSI EAESSSLTSVTTEETKVPMPAHIAVASSTTKGLIARKEGRYREPPPTPPGYI ***** .. *****:.. ***** *****; *****;
Clone7.7 KIAA0313	GIPIADFPEGCHPARKPPDYNVALQSRMVARPTEAPAG--QTPP-AAAASRPGSKPQ GIPITDFPEGHSHPARKPPDYNVALQSRMVARSSTAGPSSVQQPHGHTSSSRPVNKPQ ****; ***** . ***** *****:..* * * * * . ***
Clone7.7 KIAA0313	WHKPSDADPRLAPFQAGFAGAEDEDEQVSAV WHKPNESDPRLAPYQSQGFSTEDEDEQVSAV ****.: *****; * : *****

Figure 3A Schematic Diagram of GRF4



4618 b.p.

1/1 31/11
 atg aaa cca cta gca atc cca gct aac cat gga gtt atg ggc cag cag gag aaa cac tca
 M K P L A I P A N H G V M G Q Q E K H S
 61/21 91/31
 ctt cct gca gat ttc aca aaa ctg cat ctt act gac agt ctc cac cca cag gtg acc cac
 L P A D F T K L H L T D S L H P Q V T H
 121/41 151/51
 gtt tct tct agc cat tca gga tgt agt atc act agt gat tct ggg agc agc agt ctt tct
 V S S S H S G C S I T S D S G S S S L S
 181/61 211/71
 gat atc tac cag gcc aca gaa agc gag gct ggt gat atg gac ctg agt ggg ttg cca gaa
 D I Y Q A T E S E A G D M D L S G L P E
 241/81 271/91
 aca gca gtg gat tcc gaa gac gac gat gaa gaa gac att gag aga gca tca gat cct
 T A V D S E D D D E E D I E R A S D P
 301/101 331/111
 ctg atg agc agg gac att gtg aga gac tgc cta gag aag gac cca att gac cgg aca gat
 L M S R D I V R D C L E K D P I D R T D
 361/121 391/131
 gat gac att gaa caa ctc ttg gaa ttt atg cac cag ttg cct gct ttt gcc aat atg aca
 D D I E Q L L E F M H Q L P A F A N M T
 421/141 451/151
 atg tca gtg agg cga gaa ctc tgt gct gtg atg gtg ttc gca gtg gtg gaa aga gca ggg
 M S V R R E L C A V M V F A V V E R A G
 481/161 511/171
 acc ata gtg tta aat gat ggt gaa gag ctg gac tcc tgg tca gtg att ctc aat gga tct
 T I V L N D G E E L D S W S V I L N G S
 541/181 571/191
 gtg gaa gtg act tat cca gat gga aaa gca gaa ata ctg tgc atg gga aat agt ttt ggt
 V E V T Y P D G K A E I L C M G N S F G
 601/201 631/211
 gtc tct cct acc atg gac aaa gaa tac atg aaa gga gtg atg aga aca aag gtg gat gac
 V S P T M D K E Y M K G V M R T K V D D
 661/221 691/231
 tgc cag ttt gtc tgc ata gcc cag caa gat tac tgc cgt att ctc aat caa gta gaa aag
 C O F V C I A Q O D Y C R I L N Q V E K
 721/241 751/251
 aac atg caa aaa gtt gaa gag gaa gga gag att gtt atg gtg aaa gaa cac cga gaa ctt
 N M Q K V E E E G E I V M V K E H R E L
 781/261 811/271
 gat cga act gga aca aga aag gga cac att gtc atc aag ggt acc tca gaa agg tta aca
 D R T G T R K G H I V I K G T S E R L T
 841/281 871/291
 atg cat ttg gtg gaa gag cat tca gta gta gat cca aca ttc ata gaa gac ttt ctg ttg
 M H L V E E H S V V D P T F I E D F L L
 901/301 931/311
 acc tat agg act ttt ctt tct agc cca atg gaa gtg ggc aaa aag tta ttg gag tgg ttt
 T Y R T F L S S P M E V G K K L L E W F
 961/321 991/331
 aat gac ccg agc ctc agg gat aag gtt aca cgg gta gta tta ttg tgg gta aat aat cac
 N D P S L R D K V T R V V L L W V N N H
 1021/341 1051/351
 ttc aat gac ttt gaa gga gat cct gca atg act cga ttt tta gaa gaa ttt gaa aac aat
 F N D F E G D P A M T R F L E E F E N N

cNMP-BD

REM

(continued next page)

Figure 3B

4/34

1081/361 1111/371
 ctg gaa aga gag aaa atg ggt gga cac cta agg ctg ttg aat atc gcg tgt gct gct aaa
L E R E K M G G H L R L L N I A C A A K

1141/381 1171/391
 gca aaa aga ttg atg acg tta aca aaa cca tcc cga gaa gct cct ttg cct ttt atc
A K R R L M T L T K P S R E A P L P F I

1201/401 1231/411
 tta ctt gga ggc tct gag aag gga ttt gga atc ttt gtt gac agt gta gat tca ggt agc
L L G G S E K G F G I F V D S V D S G S

1261/421 1291/431
 aaa gca act gaa gca ggc ttg aaa cgg ggg gat cag ata tta gaa gta aat ggc caa aac
K A T E A G L K R G D O I L E V N G O N

1321/441 1351/451
 ttt gaa aac att cag ctg tca aaa gct atg gaa att ctt aga aat aac aca cat tta tct
F E N I Q L S K A M E I L R N N T H L S

1381/461 1411/471
 atc act gtg aaa acc aat tta ttt gta ttt aaa gaa ctt cta aca aga ttg tca gaa gag
I T V K T N L F V F K E L L T R L S E E

1441/481 1471/491
 aaa aga aat ggt gcc ccc cac ctt cct aaa att ggt gac att aaa aag gcc agt cgc tac
K R N G A P H L P K I G D I K K A S R Y

1501/501 1531/511
 tcc att cca gat ctt gct gta gat gta gaa cag gtg ata gga ctt gaa aaa gtg aac aaa
S I P D L A V D V E Q V I G L E K V N K

1561/521 1591/531
 aaa agt aaa gcc aac act gtg gga gga agg aac aag ctg aaa aag ata ctc gac aag act
K S K A N T V G G R N K L K K I L D K T

1621/541 1651/551
 cgg atc agt atc ttg cca cag aaa cca tac aat gat att ggg att ggt cag tct caa gat
R I S I L P Q K P Y N D I G I G Q S Q D

1681/561 1711/571
 gac agc ata gta gga tta agg cag aca aag cac atc cca act gca ttg cct gtc agt gga
D S I V G L R Q T K H I P T A L P V S G

1741/581 1771/591
 acc tta tca tcc agt aat cct gat tta ttg cag tca cat cat cgc att tta gac ttc agt
T L S S S N P D L L Q S H H R I L D F S

1801/601 1831/611
 gct act cct gac ttg cca gat caa gtg cta agg gtt ttt aag gct gat cag caa agc cgc
A T P D L P D Q V L R V F K A D Q Q S R

1861/621 1891/631
 tac atc atg atc agt aag gac act aca gca aag gaa gtg gtc att cag gct atc agg gag
Y I M I S K D T T A K E V V I Q A I R E

1921/641 1951/651
 ttt gct gtt act gcc acc ccg gat caa tat tca cta tgt gag gtc tct gtc aca cct gag
F A V T A T P D Q Y S L C E V S V T P E

1981/661 2011/671
 gga gta atc aaa caa aga aga ctt cca gat cag ctt tcc aaa ctt gca gac aga ata caa
G V I K Q R R L P D Q L S K L A D R I Q

2041/681 2071/691
 ctg agt gga agg tat tat ctg aaa aac aac atg gaa aca gaa act ctt tgt tca gat gaa
L S G R Y Y L K N N M E T E T L C S D E

2101/701 2131/711
 gat gct cag gag ttg ttg aga gag agt caa att tcc ctc ctt cag ctc agc act gtg gaa
D A Q E L L R E S Q I S L L Q L S T V E

2161/721 2191/731
 gtt gca aca cag ctc tct atg cga aat ttt gaa ctc ttt cgc aac att gaa cct act gaa
V A T O L S M R N F E L F R N I E P T E

PDZ

RA

(continued next page)

5/34

2221/741 2251/751
 tat ata gat tta ttt aaa ctc aga tca aaa acc agc tgt gcc aac ctg aag aga ttt
Y I D D L F K L R S K T S C A N L K R F
 2281/761 2311/771
 gaa gaa gtc att aac cag gaa aca ttt tgg gta gca tct gaa att ctc aga gaa aca aac
E E V I N Q E T F W V A S E I L R E T N
 2341/781 2371/791
 cag ctg aag agg atg aag atc att aag cat ttc atc aag ata gca ctg cac tgt agg gaa
Q L K R M K I I K H F I K J A L H C R E
 2401/801 2431/811
 tgc aag aat ttt aac tca atg ttt gca atc atc agt ggc cta aac ctg gca cca gtg gca
C K N F N S M F A I I S G L N L A P V A
 2461/821 2491/831
 aga ctg cga acg acc tgg gag aaa ctt ccc aat aaa tac gaa aaa cta ttt caa gat ctc
R L R T T W E K L P N K Y E K L F Q D L
 2521/841 2551/851
 caa gac ctg ttt gat cct tcc aga aac atg gca aaa tat cgt aat gtt ctc aat agt caa
Q D L F D P S R N M A K Y R N V L N S Q
 2581/861 2611/871
 aat cta caa cct ccc ata atc cct cta ttc cca gtt atc aaa aag gat ctc acc ttc ctt
N L Q P P I I P L F P V I K K D L T F L
 2641/881 2671/891
 cac gaa gga aat gac tca aaa gta gac ggg ctg gtc aat ttt gag aag cta agg atg att
H E G N D S K V D G L V N F E K L R M I
 2701/901 2731/911
 gca aaa gaa att cgt cac gtt ggc cga atg gct tca gtg aac atg gac cct gcc ctc atg
A K E I R H V G R M A S V N M D P A L M
 2761/921 2791/931
 ttc agg act cgg aag aag aaa tgg cgg agt ttg ggg tct ctc agc cag ggt agt aca aat
F R T R K K K W R S L G S L S Q G S T N
 2821/941 2851/951
 gca aca gtg cta gat gtt gct cag aca ggt ggt cat aaa aag cgg gta cgt cgt agt tcc
A T V L D V A Q T G G H K K R V R R S S
 2881/961 2911/971
 ttt ctc aat gcc aaa aag ctt tat gaa gat gcc caa atg gct cga aaa gtg aag cag tac
F L N A K K L Y E D A Q M A R K V K Q Y
 2941/981 2971/991
 ctt tcc aat ttg gag cta gaa atg gac gag gag agt ctt cag aca tta tct ctg cag tgt
L S N L E L E M D E E S L Q T L S L Q C
 3001/1001 3031/1011
 gag cca gca acc aac aca ttg cct aag aat cct ggt gac aaa aag cct gtc aaa tcc gag
E P A T N T L P K N P G D K K P V K S E
 3061/1021 3091/1031
 acc tct cca gta gct cca agg gca ggg tca caa cag aaa gct cag tcc ctg cca cag ccc
T S P V A P R A G S Q Q K A Q S L P Q P
 3121/1041 3151/1051
 cag cag cag cca cca cca gca cat aaa atc aac cag gga cta cag gtt ccc gcc gtg tcc
Q Q Q P P P A H K I N Q G L Q V P A V S
 3181/1061 3211/1071
 ctt tat cct tca cgg aag aaa gtg ccc gta aag gat ctc cca cct ttt ggc ata aac tct
L Y P S R K K V P V K D L P P F G I N S
 3241/1081 3271/1091
 cca caa gct tta aaa aaa att ctt tct ttg tct gaa gaa gga agt ttg gaa cgt cac aag
P Q A L K K I L S L S E E G S L E R H K
 3301/1101 3331/1111
 aaa cag gct gaa gat aca ata tca aat gca tct tgg cag ctt tct tct cct cct act tct
K Q A E D T I S N A S S Q L S S P P T S

CDC25

Insertion
Unique to
GRF4

Continuation of the
CDC25 domain

(continued next page) 6/34

3361/1121 3391/1131
 cca cag agt tct cca agg aaa ggc tat act ttg gct ccc agt ggt act gtg gat aat ttt
 P Q S S P R K G Y T L A P S G T V D N F
 3421/1141 3451/1151
 tca gat tct ggt cac agt gaa att tct tca cga tcc agt att gtt agc aat tcg tct ttt
 S D S G H S E I S S R S S I V S N S S F
 3481/1161 3511/1171
 gac tca gtg cca gtc tca ctg cac gat gag agg cgc cag agg cat tct gtc agc atc gtg
 D S V P V S L H D E R R Q R H S V S I V
 3541/1181 3571/1191
 gaa aca aac cta ggg atg ggc agg atg gag agg cgg acc atg att gaa cct gat cag tat
 E T N L G M G R M E R R T M I E P D Q Y
 3601/1201 3631/1211
 agc ttg ggg tcc tat gca cca atg tcc gag ggc cga ggc tta tat gct aca gct aca gta
 S L G S Y A P M S E G R G L Y A T A T V
 3661/1221 3691/1231
 att tct tct cca agc aca gag gaa ctt tcc cag gat cag ggg gat cgc gcg tca ctt gat
 I S S P S T E E L S Q D Q G D R A S L D
 3721/1241 3751/1251
 gct gct gac agt ggc cgt ggg agc tgg acg tca tgc tca agt ggc tcc cat gat aat ata
 A A D S G R G S W T S C S S G S H D N I
 3781/1261 3811/1271
 cag acg atc cag cac cag aga agc tgg gag act ctt cca ttc ggg cat act cac ttt gat
 Q T I Q H Q R S W E T L P F G H T H F D
 3841/1281 3871/1291
 tat tca ggg gat cct gca ggt tta tgg gca tca agc agc cat atg gac caa att atg ttt
 Y S G D P A G L W A S S S H M D Q I M F
 3901/1301 3931/1311
 tct gat cat agc aca aag tat aac agg caa aat caa agt aga gag agc ctt gaa caa gcc
 S D H S T K Y N R Q N Q S R E S L E Q A
 3961/1321 3991/1331
 cag tcc cga gca agc tgg gcg tct tcc aca ggt tac tgg gga gaa gac tca gaa ggt gac
 Q S R A S W A S S T G Y W G E D S E G D
 4021/1341 4051/1351
 aca ggc aca ata aag cgg agg ggt gga aag gat gtt tcc att gaa gcc gaa agc agt agc
 T G T I K R R G G K D V S I E A E S S S
 4081/1361 4111/1371
 cta acg tct gtg act acg gaa gaa acc aag cct gtc ccc atg cct gcc cac ata gct gtg
 L T S V T T E E T K P V P M P A H I A V
 4141/1381 4171/1391
 gca tca agt act aca aag ggg ctc att gca cga aag gag ggc agg tat cga gag ccc ccg
 A S S T T K G L I A R K E G R Y R E P P
 4201/1401 4231/1411
 ccc acc cct ccc ggc tac att gga att ccc att act gac ttt cca gaa ggg cac tcc cat
 P T P P G Y I G I P I T D F P E G H S H
 4261/1421 4291/1431
 cca gcc agg aaa ccg ccg gac tac aac gtg gcc ctt cag aga tcg cgg atg gtc gca cga
 P A R K P P D Y N V A L Q R S R M V A R
 4321/1441 4351/1451
 tcc tcc gac aca gct ggg cct tca tcc gta cag cag cca cat ggg cat ccc acc agc agc
 S S D T A G P S S V Q Q P H G H P T S S
 4381/1461 4411/1471
 agg cct gtg aac aaa cct cag tgg cat aaa ccg aac gag tct gac ccg cgc ctc gcc cct
 R P V N K P Q W H K P N E S D P R L A P

PY motifs

(continued next page)

7/34

4441/1481

4471/1491

tat cag tcc caa ggg ttt tcc acc gag gag gat gaa gat gaa caa gtt tct gct gtt tga
Y Q S Q G F S T E E D E D E Q V S A V *

4501/1501

4531/1511

PDZ binding motif

ggc aca gac ttt tct gga agc aga gcg agc cac ctg aaa gga gag cac aag aag acg tcc
G T D F S G S R A S H L K G E H K K T S

4561/1521

4591/1531

tga gca ttg gag cct tgg aac tca cat tct gag gac ggt gga cca gtt tgc ctc ctt c
* A L E P W N S H S E D G G P V C L L

CDC25 Domain

hGRF4 SQISLLQLSTVEVATQLSMRNFELFRNIEPTEYIDDLF---KLRSKTSCANLKRFEVIN
dGRF4 SNVHFLHLNAYELAIQTLTLODFANFRQIESTEYVDELF---ELRSRYGVPMLSKEAELVN
hEpac SAEGLDLVSADLAGQLTDHDSLFNSIHQVELIHVVLGPOHLRDVT-TANLERFMRRFN
mRasGRF2 KAECFETLSAMELAEQITLLDHIVFRSIPYEEFLGQGW--MKLDKNERTPYIMKTSQHFN
dSOS DEITLLTLHPLELARQLTLLEFEMYKNVKPSELVGSPT--KKDKVKSPNLLKMKHTT
hRasGRP VSLLEFDHLEPEELSEHLTYLEFKSFRRISFSQYQNYLVN----SCVKENPTMERIALCN
: : . : : : : : . : : .

hGRF4 QETFWVASEILRETNQLKRMKIIKHFIKIALHCRECKNFNSMFIIISGLNLAPVARLRTT
dGRF4 REMFWVSEICAENIVRRMKIVKQFIKIAHCKEKNFNSMFIVSGLGHGAVSRLRQT
hEpac ELQYWVATELCCLPVPGPRAQLLRKFIKLAHLKEQKNLSFFAVMFGLSNSAISRLAHT
mRasGRF2 EMSNLVASQIMNYADISSRPNAIEKWAVADICRCLHNYNGVLEITSALNRSPIYRLKKT
dSOS NVTRWIEKSITEAENYEERLAIMQRAIEVMMVHLELNNFNGLSIVAAMGTASVYRLRWT
hRasGRP GISQWVQLMVLRSPTQLRAEVFIKFIQVAQKLHQLQNFNTLMAVIGGLCHSSISRLKET
: : * . : : : . * * : : . : . : * *

hGRF4 WEKLPNKYEKLFQDLQDLFDPNMAKYRNVLSQNL-QPPIIPLFPVIKKDLTFLEHGN
dGRF4 WEKLPNPKYQRLFNDLQDLMDPSRNSKYRQLVSAELLAQHPIIPFYPIVKKDLTFIHLGN
hEpac WERLPHKVRKLYSALERLLDPSWNHRVYR-LALAKLS--PPVIPFMPLLLKDMTFIHEGN
mRasGRF2 WAKVSKQTKALMDKLQKTVSSEGRFKNLR-ETLKNCN--PPAVPYLGMYLTDLAFIEEGT
dSOS FOGLPERYRKFLKEECRELSDDHLKKYQER--L-RSIN--PPCVPFGRYLTNHLHEEGN
hRasGRP SSHVPHEINKVLGEMTELLSSSRNYDNYR-RAYGECT--DFKIPILGVHLKDLISLYEAM
: * . : * : : .

hGRF4 DSKVDG--LVNFEKLRMIAKEIRHVGRMASVNMDPALMFRTTRKKK---WRSLSLSQGS
dGRF4 DTRVDG--LINFELKRLAKEVRLTHMCSSPYDLLSILELKGQSPSNALFSLNQMSASQ
hEpac HTLVEN--LINFELKRMMAAARMHLHCRSHNPVPLSPLRSRVSHLHEDSQVARISTCSE
mRasGRF2 PNFTEEG-LVNFSKMRMISHIIREIRQFQQTAYRIDQQP-----
dSOS PDLLANTELINFSKRRKVAEIIIGEIQYQONQPYCLNEES-----
hRasGRP PDYLEDG-KVNVHKLALYNHISELVQLQEVAPPLEANKDL-----
: * . : : .

hGRF4 TNATVLVDVAQTGG---HKKRVRSSFLNAKKLYEDAQMARKVKQYLSNLELEMDDESLQ
dGRF4 SNAAAGTVIAANAGQATIKRRKKSTAAPNPKMFEEAQMVRRVKAYLSNLKILSDEDLLH
hEpac -----QSLSTRSPASTWAYVQQLKVIDNQRELS
mRasGRF2 -----KVIQYLLDKALVIDEDSLY
dSOS -----TIRQFFEQLDPFNGLSDKQMSDYLY
hRasGRP -----VHLLTSLDLYYTEDEIY
: :

Figure 4

hGRF4 TSLQCEPATNT
dGRF4 KFSLECEPAHGS
hEpac RLSRELEP----
mRasGRF2 ELSLKIEPRLPA
dSOS NESLRIEPRGCK
hRasGRP ELSYAREPRNHR
* **

GRF4-REM domain

Figure 5:

CDC25
 Sos_mouse_
 RasGEF_aimless_
 GRF2_mouse_
 GRF4

-IRGGTKEALIEHLT-SHELVDAAFNVMTLITFRSILT-TREFFYALIYRY-
 -IKGGTVVKLIERT-YHMYADPNF-VRTFLTYRSFCK-PQELLNLLIERFE
 VVKFASLNKLVEHLT-HDSKHDQLQFLKTFMLTYQSFCF-PEKLMSKLQORY-
 -IRYASVEALLERLT-DLRFSLIDFLNTFLHTYRIFTT-ATVVLAKLSDIY-
 -IKG-TSERLTMHLVEHSVVDPTFIEDFLTYRTFLSSPMEVGKLLLEWFN
 :: : * :*. . * :*:: : . . . * :

Figure 6A:

Overall structure comparison between
GRF4 and other known mammalian

RasGEF/RasGRF

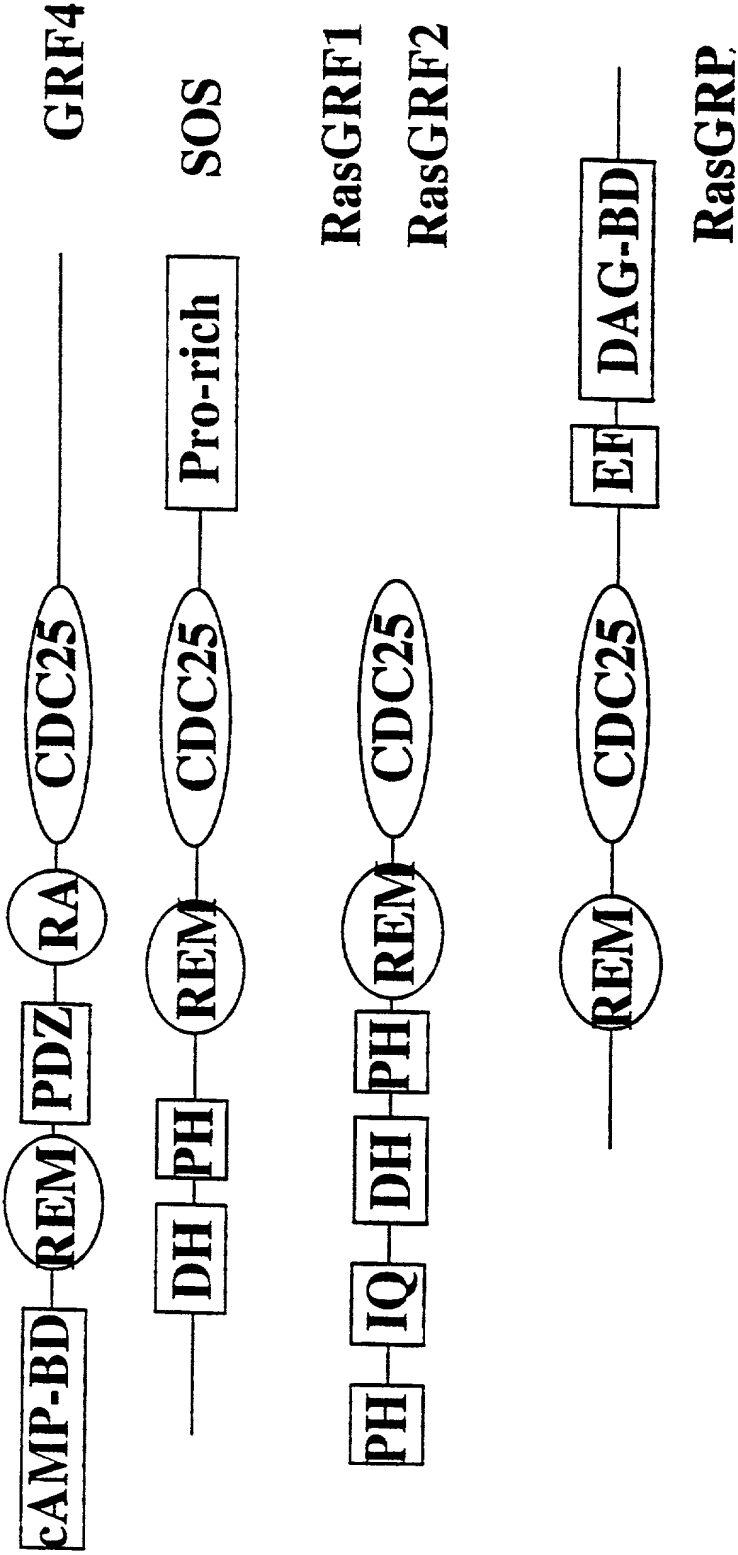
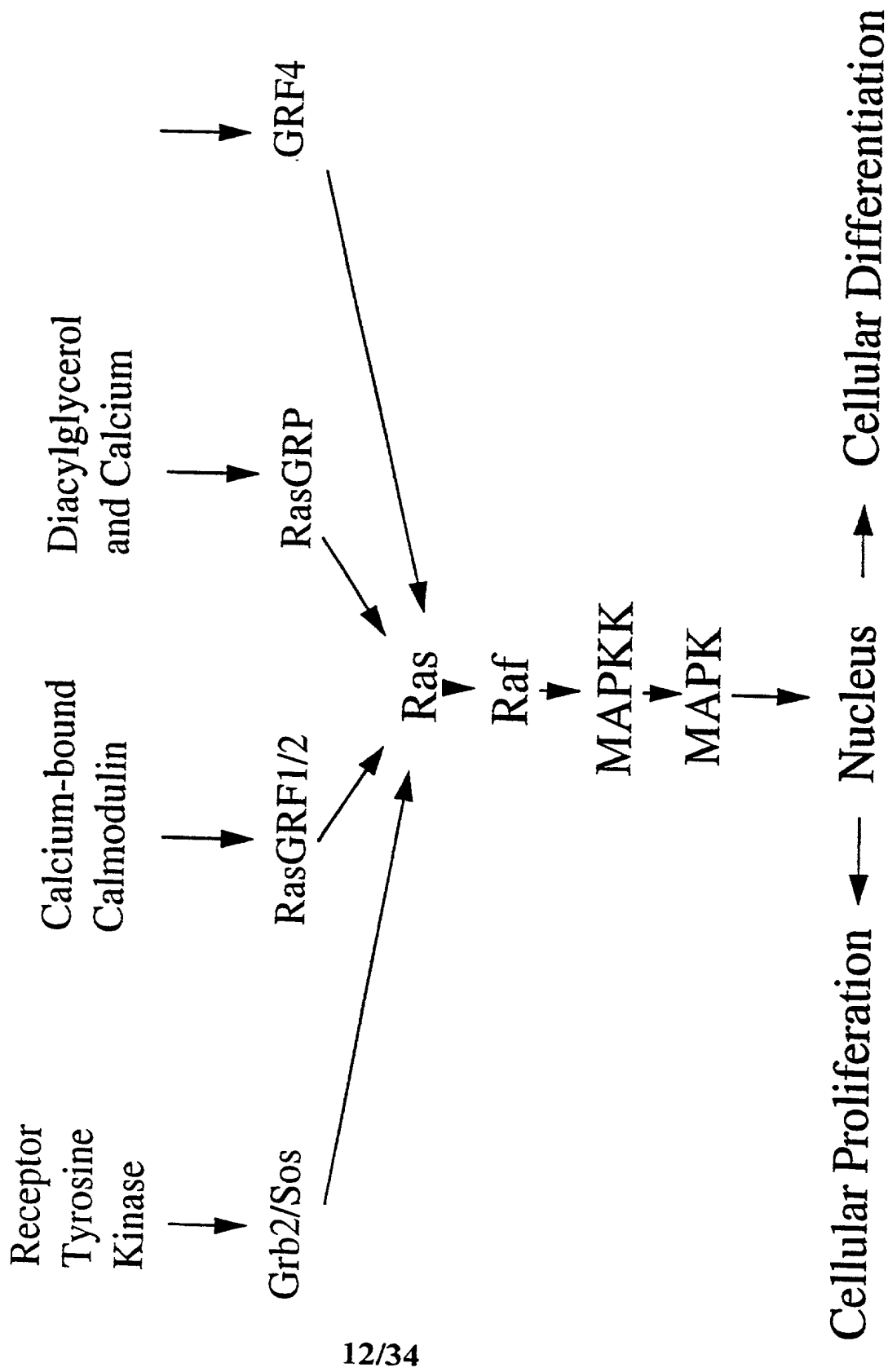


Fig.6B

Ras Signaling pathway



12/34

100220" 9287660

Figure 7

PDZ domain

hGRF4	LTKPSREAPLPFILLGSEK-----GFGIFVDSVDSGSKATEAG-LKRGDQIL
dGRF4	LTRSSRDEPLNFRIVGGYELRGVAIATGNAAVGIYISHVEPGSKAQDVG-LKRGDQIH
hPTP-BAS-1	NLKKDAKYGLGFQIIGGEKMGRL-----DLGIFISSVAPGPGPADLDGCLKPGDRLI
hPSD-95	IVIHRRGSTGLGFNIVGGEDG-----EGIFISFILAGGPADLSGELRKGDQIL
rLin-7-C	VELPKTEEGLGFNIMGKEQ-----NSPIYISRIIPGGIADRHGGLKRGDQLL
hDGL	VKVQKGSEPLGISIVSGEKG-----GIYVSKVTVGSIAHQAG-LEYGDQLL
	. * : : : * . * : : : * : : : * : : : * : : : *
hGRF4	EVNGQNFENIQLSKAMEILR
dGRF4	EVNGQSLDHVTSKRALEILT
hPTP-BAS-1	SVNSVSLEGVSHHAAIEILQ
hPSD-95	SVNGVDLNRNASHEQAAIALK
rLin-7-C	SVNGVSVEGEHHEKAVELLK
hDGL	EFNGINLRSATEQQARLIIG
	..* . . * :

Figure 8

cNMP-BD

hGRF4
dGRF4
hEPAC
PRKAR1B
hPKGII
m-EAG

hGRF4
dGRF4
hEPAC
PRKAR1B
hPKGII
m-EAG

```

MVFAVVERAGTIVLNDGEELDSWSVILNGSVEV---TYPDGKAEILCMGNSFGVSPTMDK
MVFAVVDKAGTVVMSDGEELDSWSVLINGAVEI---EHANGSREELQMGDSFGILPTMDK
LLFEPHSAKAGTVLFSQGDKGTSWYIIWKGSVNIV--THGKGLVTTLHEGDDFGQLALVND
AMFPVTHIAGETVIOQNEGDNFYVVDQGEVDVY--VNGEWVTN-ISEGGSFGELALIYG
CMYGRNYQQGSYIIKQGEPCGNHIFVLAEGRLEV---FQGEKLLSSIPMWTTFGELAILYN
RLRSVYLPNDYVCKKGEIGREMYIIQAGQVQVLGGPDGKSVLVTLKAGSVFGEISLLAV
:      .      :      .      :      :      :      :      :      :      :
---EYMKGVMR TKVDDCQFVCIAQQDYCRILNQVEKNMQKVEEE
---LYHRGVMR TKCDDCQFVCITQTDYYRIQHQQEENTRRHEDE
---APRAATII LREDNCHFLRVDKQDFNRI IKDVEAKTMRLEEH
---TPRAATV KAKTD-LKLWGIDRDSYRRILMGSTLRKRKMYEE
---CTR TASVKAITN-VKTWALDREVFQNMRRTAQARDEQYRN
GGNRRRTANVVAHGF-TNLFILDKKDLNEILVHYPESQKLLRKK
      .      :      :      :      :      :      :      :      :      :

```

Figure 9:

GRF4-RA domain

dgk-1a_ce_ GRF4 RalGDS_h_	<pre> -----REDFE---IIRVFDGNNNS---YRSQIS-----RNIIVAKHVSQQVRDAALR --HHRILDFS---ATPDLPDQVLRVFKADQQS-----RYIMISKDTTAKEVVIQAIR SILVTSQDKAPSVISRVLKKNNRDSAVASEYELVQLLPGERELTIPASANVFYAMDGASH * : : : .: . * : : . . . * :</pre>
dgk-1a_ce_ GRF4 RalGDS_h_	<pre> RFHI--NDTPERYYT-QVVGEVEEEILED-----PVP-----LRNVKRPEGKRAQIFIR EFAV--TATPDQYSLC-EVSVTPEGVIKQ-----RLP-----DQLSK--LADRIQLSGR DFLLRHGEGPLLLHLASPVARLPQELLRVREEGAPFPGSRPQGGRLHGHCSSEEEAPLAYR * : * : * : : * . * : : *</pre>
dgk-1a_ce_ GRF4 RalGDS_h_	<pre> YVD----- YYLKNNME- SHGVHTRCG :</pre>

Figure 10

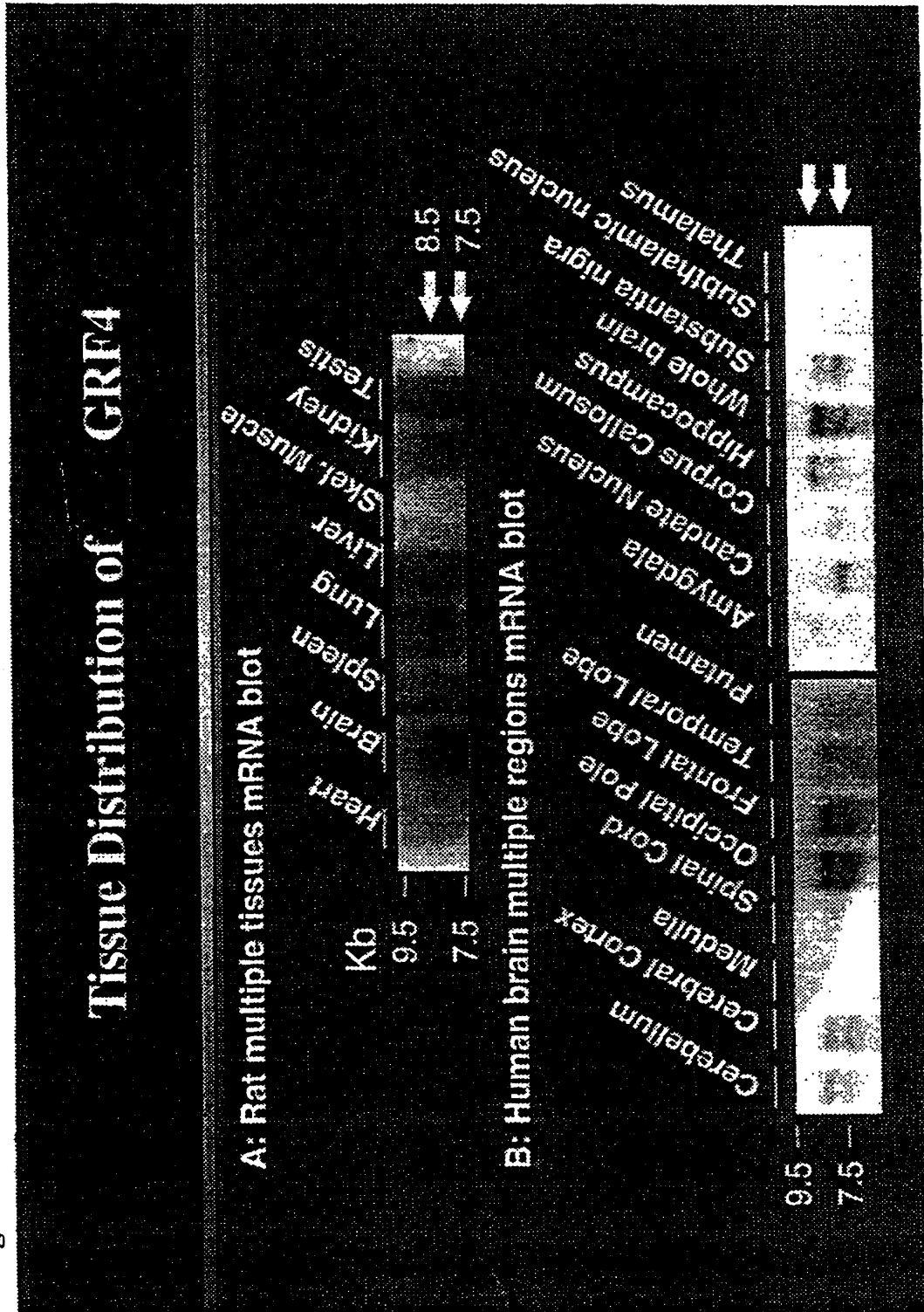


Figure 11

GST-fusion protein of the C-terminal last 150 aa of
 GRF4 containing two PY motifs pulls down
 endogenous Nedd4 in Hek 293T cells

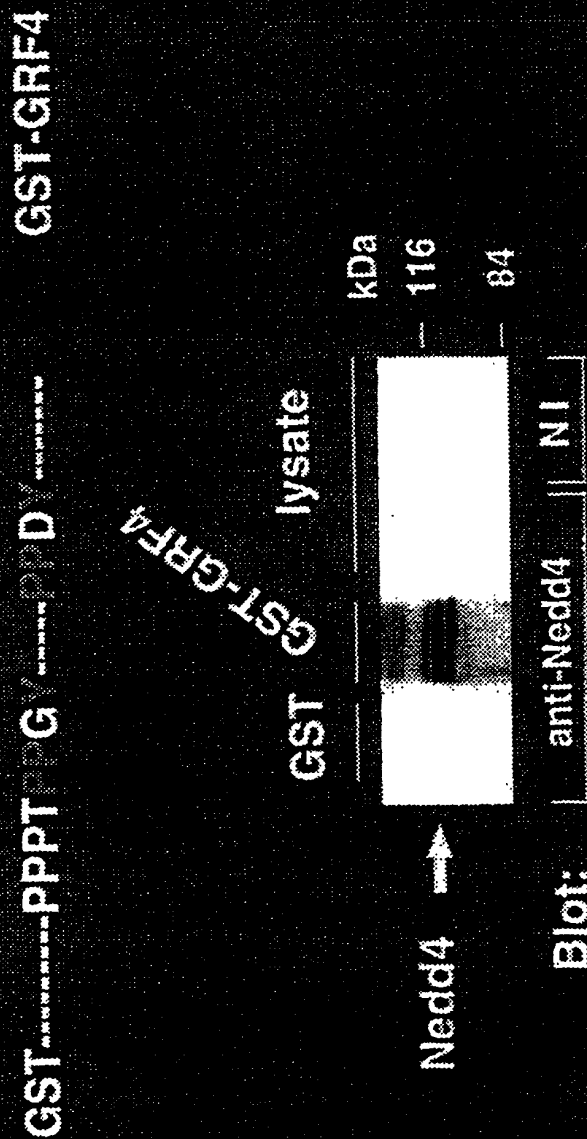


FIG. 12

Figure 12

GRF4 co-immunoprecipitates with endogenous Nedd4 in Hek 293T cells transiently transfected with Flag-tagged GRF4

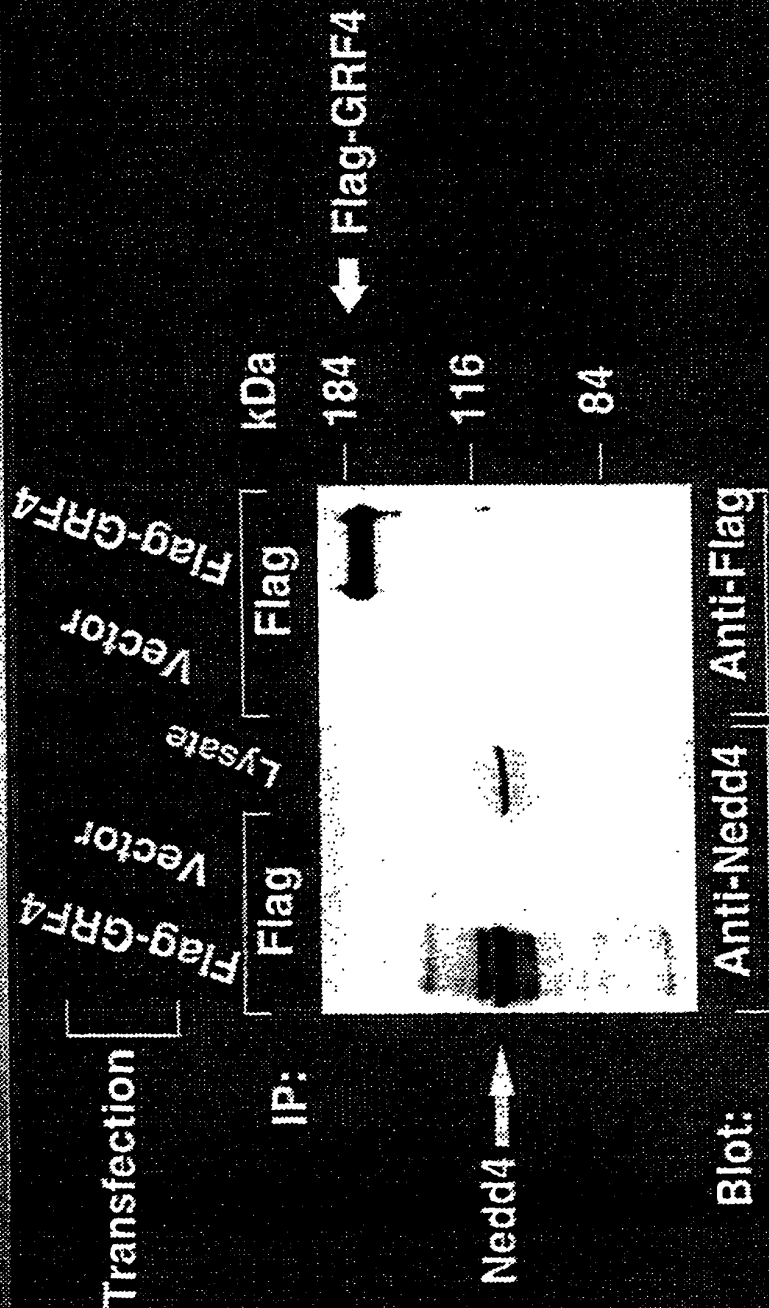


Fig.13

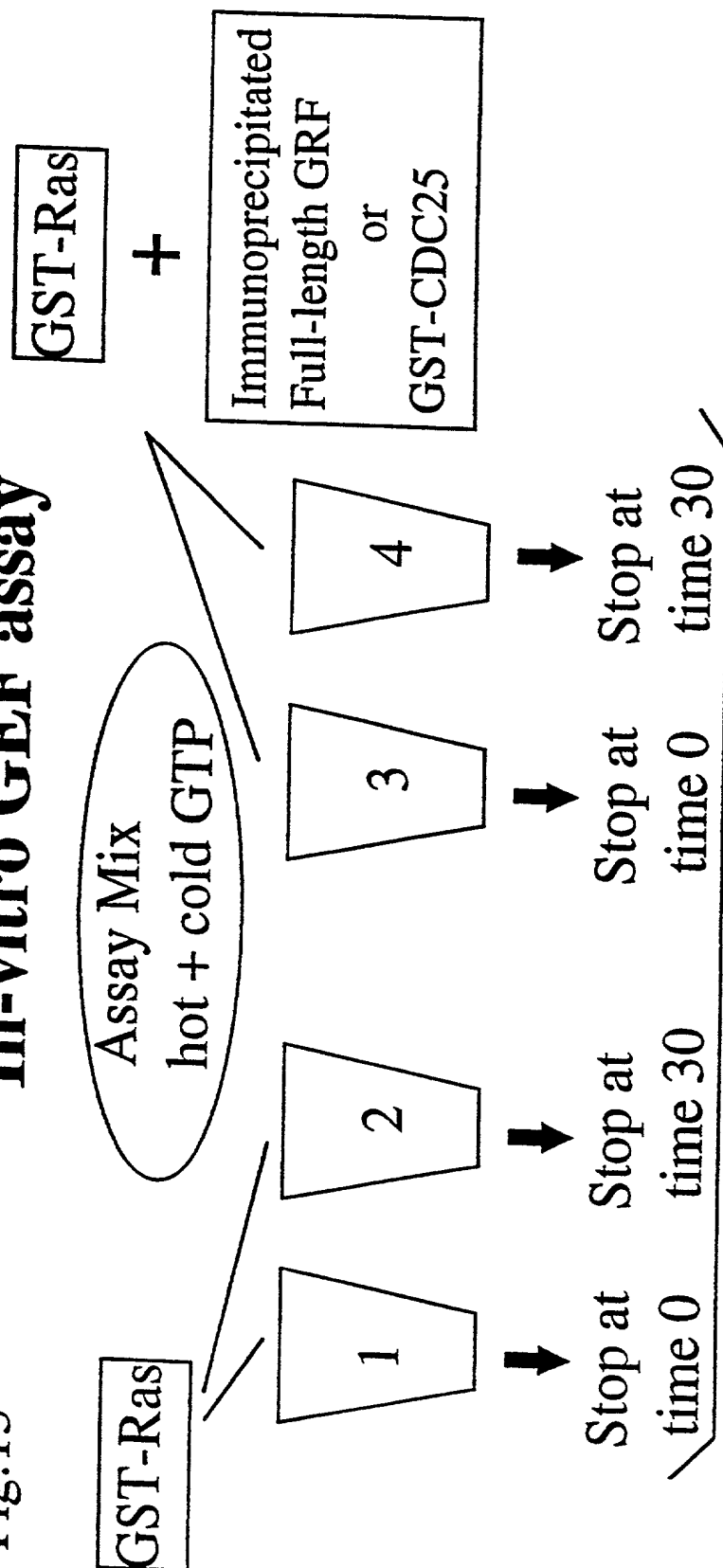
In-vitro GEF assay

FIG. 14

Fig.14 GRF4 is active on Ras : in-vitro GEF assay using immunoprecipitated full-length GRF4

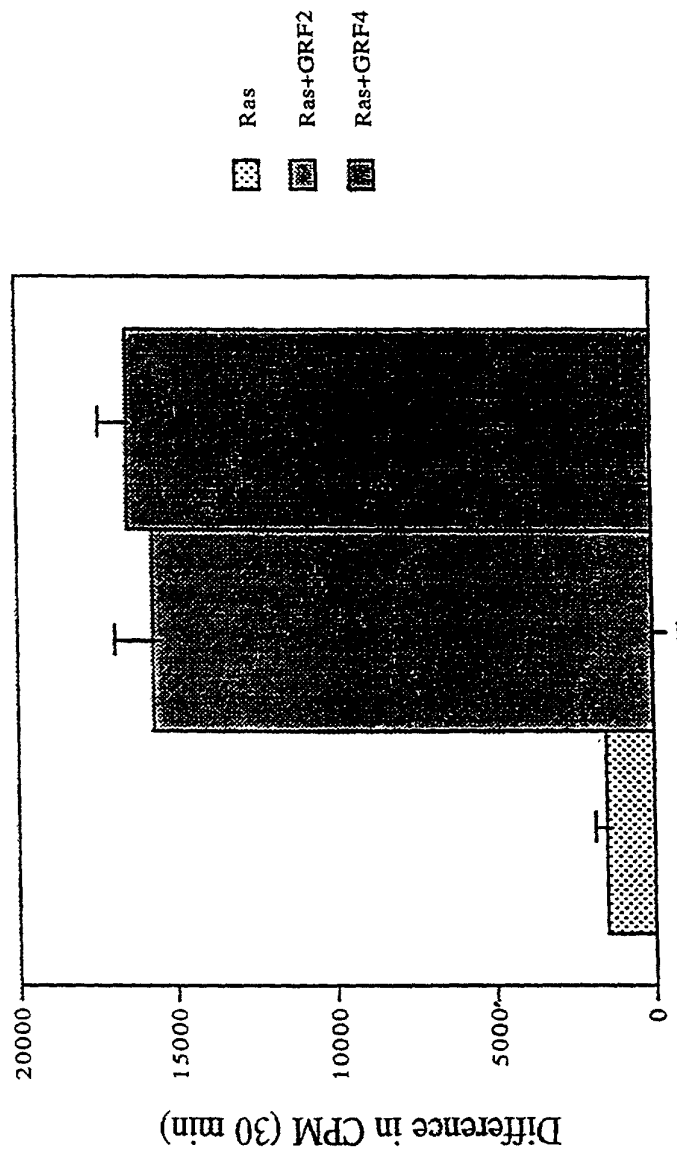


FIGURE 15

Figure 15

GRF4 forms stable complex with Ras in-vitro:
it binds preferentially to nucleotide-free and
GTP-bound Ras

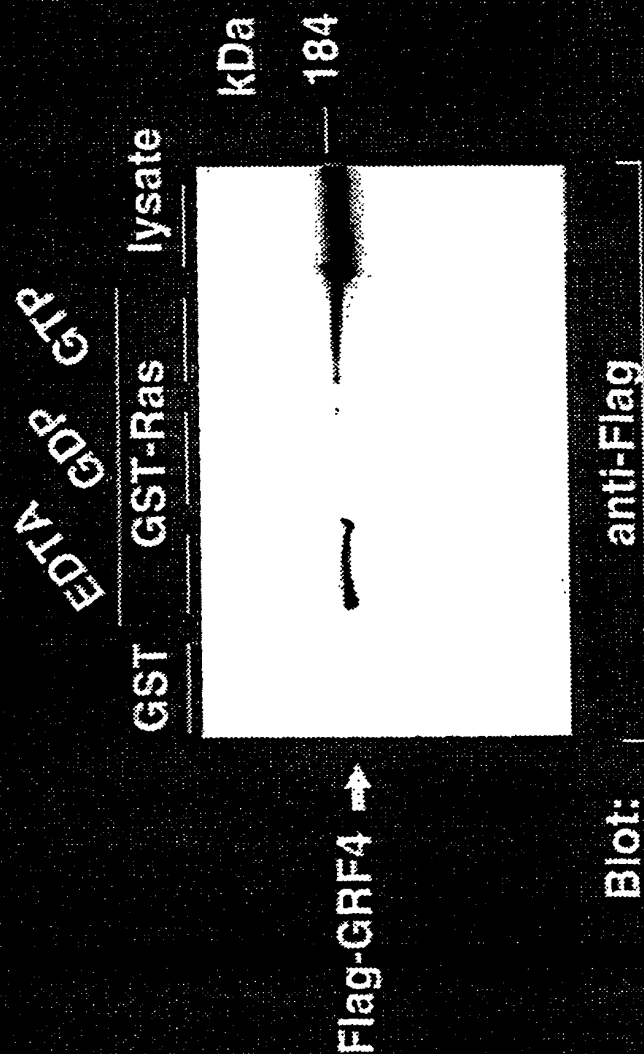
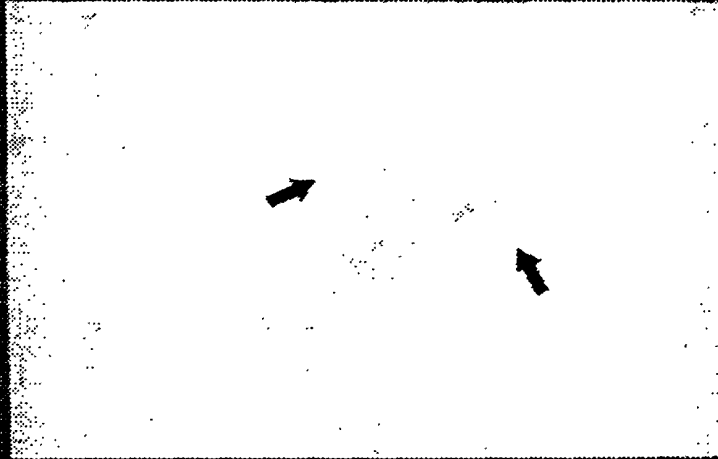


Figure 16

Transformation Assay:  GRF4 induces foci formation in Rat2 fibroblasts

RasV12



 GRF4



Empty Vector

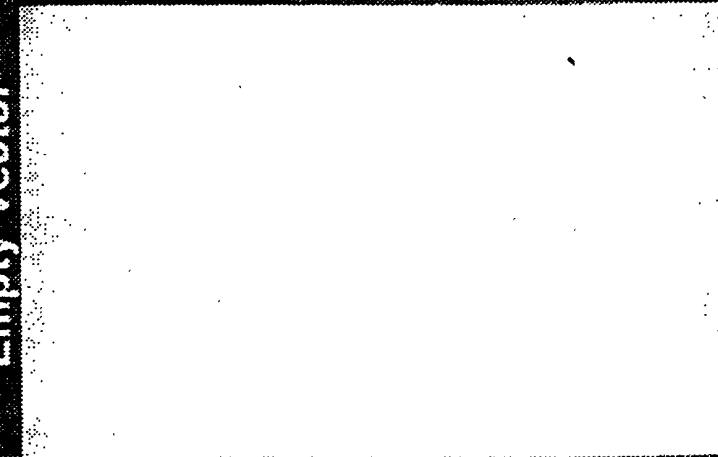
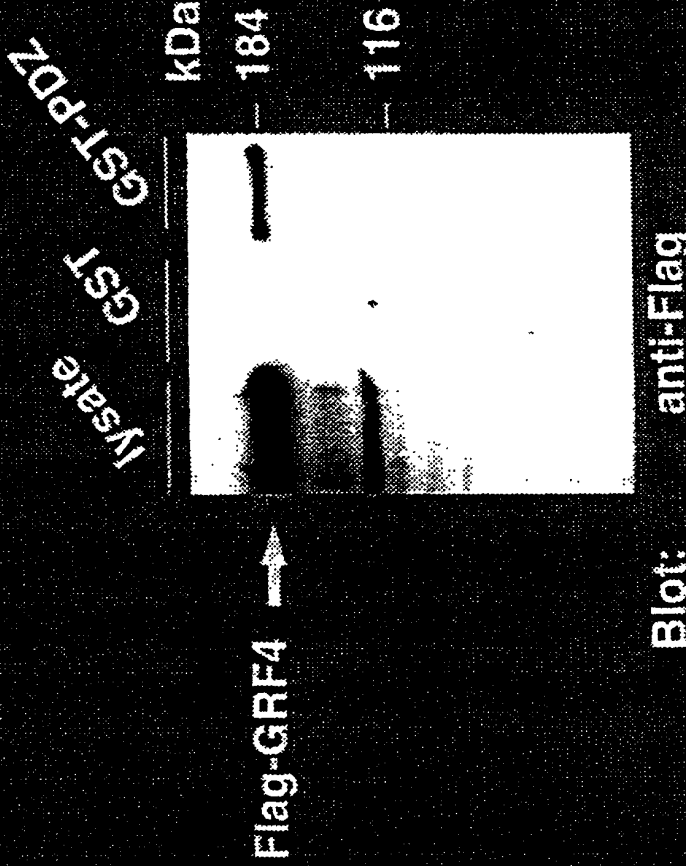


FIGURE 17

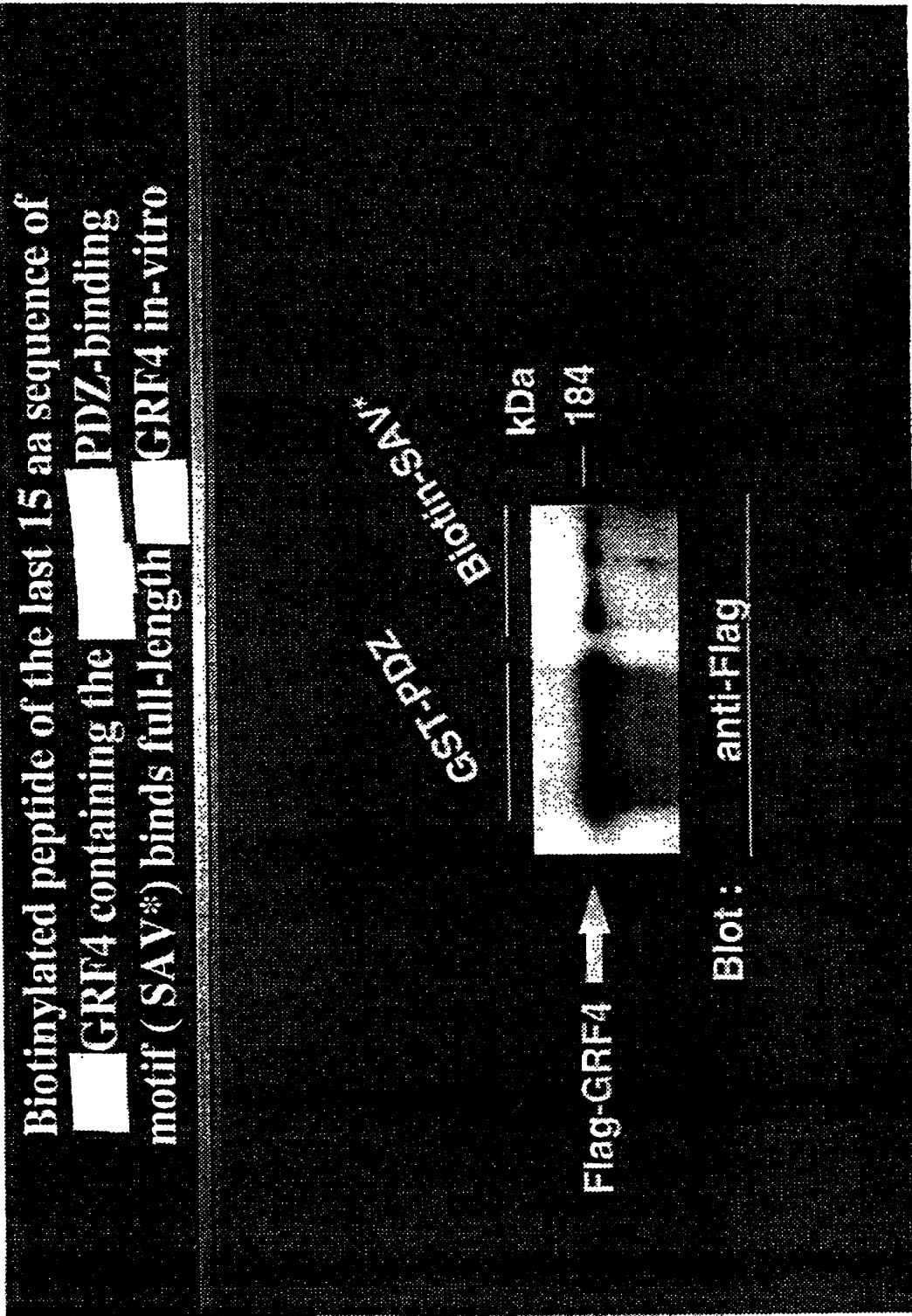
Figure 17

GST-fusion protein of  GRF4-PDZ
domain binds full-length  GRF4 in-vitro



T00220" 923TF600

Figure 18



6568 bp

/translation="MKPLAIPANHGVMGQQEKHSLPADFTKLHLTDSLHPQVTHVSSS
HSGCSITSDSGSSSLSDIYQATESEAGDMDLSGLPETA VDSEDDDDDEEDIERASDPLM
SRDIVRDCLEKDPIDRTDDIEQLLEFMHQLPAFANMTMSVRRELCAVMVFVVERAG
TIVLNDGEELDSWSVILNGSVEVTPDGKAEILCMGNSFGVSPMTMDKEYMKGVMRKTV
DDCQFVCIAQQDYCRILNQVEKNMQKVEEEGEIVMVKEHRELDRTGTRKGHIVIKGTS
ERLTMHLVEEHSVVDPTFIEDFLLTyrTFLSSPMEVGKKLLEWFNDPSLRDKVTRVVL
LWVNNHFNDFEGDPAMTRFLEEFENNLEREKMGGHLRLNIAACA AKRRLMTLTKPS
REAPLPFILLGGSEKGFIFVDSVDSGSKATEAGLKRGDQILEVNGQNFENIQLSKAM
EILRNNTLSITVKTNLFVFKELLTRLSEEKRN GAPHLPKIGDIKKASRYSIPDLAVD
VEQVIGLEKVNKSKANTVGGRNKLLKILDKTRISILPQKPYNDIGIGQSQDDSI VGL
RQTKHIPTALPVSGTLSSSNPDLLQSHHRILDFSATPDLPDQVLRVFKADQQSRYIMI
SKDTTAEVVIQAIREFAVTATPDQYSLCEVSVTPEGVIKQRRLPDQLSKLADRIQLS
GRYYLKNNMETETLCSDEDAQELLRESQISLLQLSTVEVATQLSMRNFEFRNIEPTE
YIDDLFKLRSKTSCANLKRFEVINQETFWVASEILRETNQLKRMKIIKHFIKIALHC
RECKNFNSMFAISGLNLAPVARLRTTWEKLPNKYEKLFQDLQDLDFPSRNMAYRNV
LNSQNLQPPHLPFVVIKDLTFLHEGNDKVDGLVNFELKRMIAKEIRHVGRMASVN
MDPALMFRTRKKKWRSLGSLSQGSTNA TVLDVAQTGGHKKRVRRSSFLNAKKLYEDAQ
MARKVKQYLSNLELEMDDEESLQTLSLQCEPATNTLPKNPGDKKPKVSETSPVAPRAGS
QQKAQSLPQPQQPPPAHKINQGLQVPA VSLYPSRKKVPVKDLPPFGINSPQALKKIL
SLSEEGSLERHKKQAEDTISNASSQLSSPPTSPQSSPRKGYTLAPSGTVDNFSDSGHS
EISSRSSIVSNSFSVPSLHDERRQRHSVSIVETNLGMGRMERRMTMIEPDQYSLGS
YAPMSEGRGLYATATVISSPSTEELSQDQGDRA SLDAADSGRGSWTSCSSGSHDNIQT
IQHQRSWETLPGHTHFDYSGDPAGL WASSSHMDQIMFSDHSTKYNRQNSRESLEQA
QSRASWASSTGYWGDESGDTGTIKRRGGKDV SIEAESSLTSVTTEETKVPVMPAHI
AVASSTTKGLIARKEGRYREPPPTPPGYIGIPITDFPEGHSH PARKPPDYNVALQSR
MVARSSDTAGPSSVQPHGHTSSRPVNKPQWHKP NESDPRLAPYQSQGFSTEDEDE
QVSAV" | SEQ ID NO: 2]

63. 4562

BASE COUNT 1974 a 1400 c 1463 g 1731 t
ORIGIN

1 ctgccatcg tgagagattg gtacatgatg tgtaaatca gttcagcata tgtttctca
61 ttaigaaacc actagcaatc ccagctaacc atggagttat gggccagcag gagaacact
121 cacttctcgc agatttcaca aaactgcatc ttactgacag tctccacca caggtgaccc
181 acgtttctc tagccattca ggatgtagta tcactagtag ttctgggagc agcagctctt
241 ctgatatcta ccaggccaca gaaagcgagg ctggtgatat ggacctgagt ggggtgccag
301 aaacagcagt ggattccgaa gacgacgacg atgaagaaga cattgagaga gcatcagatc
361 ctctgatgag cagggacatt gtgagagact gcctagagaa ggaccaat gaccggacag
421 atgatgacat tgaacaactc ttggaattta tgcaccagtt gcctgctttt gccaatatga
481 caatgtcagt gaggcgagaa ctctgtgctg tgatggtgtt cgcagtgggt gaaagagcag
541 ggaccatagt gttaatgat ggtgaagagc tggactcctg gtcagtgatt ctcaatggat
601 ctgtggaagt gacttatcca gatgaaaag cagaaatact gtgcatggga aatagttttg
661 gtgtctctcc taccatggac aaagaataca tgaaggagat gatgagaaca aaggtggatg
721 actgccagtt tgtctgcata gccagcaag attactgccg tattctcaat caagtagaaa
781 agaacatgca aaaagttaga gaggaaggag agattgttat ggtgaaagaa caccgagaac
841 ttgatcgaa tggaacaaga aaggacaca ttgatcaaa ggttaccca gaaagggtta
901 caatgcattt ggtggaagag cattcagtag tagatccaac attcatagaa gactttctgt
961 tgacctatag gactttctt tctagcccaa tggaagtggg caaaaagtta ttggagtgtt
1021 ttaatgacct gagcctcagg gataaggta cacgggtagt attattgttg gtaataatc
1081 acttcaatga cttgaagga gatcctgcaa tgactcgatt tttagaagaa ttgaaaaca
1141 atctggaaag agagaaaatg ggtggacacc taaggctgtt gaatatcgcg tgtgctgcta
1201 aagcaaaaag aagattgatg acgttaaca aaccatcccg agaagctcct ttgcctttta
1261 tcttacttgg aggctctgag aagggtttg gaatcttgt tgacagtga gattcaggta
1321 gcaaaagcaac tgaagcaggc tgaacaggg gggatcagat attagaagta aatggccaaa
1381 acctgaaaa cattcagctg tcaaaagcta tggaaattct tagaataac acacatttat

(continued next page)

Figure 19A

1441 ctatcactgt gaaaaccaat ttattgtat ttaaagaact tctaacaaga ttgtcagaag
 1501 agaaaaagaaa tgggtccccc caccttccta aaattggtga cattaanaag gccagtcgct
 1561 actccattcc agatcttctg gtagatgtag aacaggtgat aggactgaa aaagtgaaca
 1621 aaaaaagtaa agccaacact gtgggaggaa ggaacaagct gaaaaagata ctgcacaaga
 1681 ctggatcag tatcttgcca cagaaccat acaatgatat tgggattggt cagtctcaag
 1741 atgacagcat agtaggatta aggcagacaa agcacatccc aactgcattg cctgtcagtg
 1801 gaaccttacc atccagtaat cctgatttat tgcagtcaca tcatcgcat ttgacttca
 1861 gtgctactcc tgacttgcca gatcaagtgc taagggtttt taaggctgat cagcaaaagcc
 1921 gctacatcat gatcagtaag gacactacag caaaggaagt ggtcattcag gctatcaggg
 1981 agttgtctgt tactgccacc cggatcaat attactatg tgaggtctct gtcacacctg
 2041 agggagtaat caaacaaga agacttcag atcagcttc caaactgca gacagaatac
 2101 aactgagtg aaggtattat ctgaaaaaca acatggaaac agaaactctt tgttcagatg
 2161 aagatgctca ggagttgttg agagagagtc aaattccct cctcagctc agcactgtgg
 2221 aagttgcaac acagctctct atgcgaaatt ttgaactctt tcgcaacatt gaacctactg
 2281 aatatataga tgatttttt aaactcagat caaaaaccag ctgtgccaac ctgaagagat
 2341 ttgaagaagt cattaaccag gaaacatttt gggtagcatc tgaattctc agagaacaa
 2401 accagctgaa gaggatgaag atcattaagc attcatcaa gatagcactg cactgtaggg
 2461 aatgcaagaa tttaactca atgttgcaa tcatcagtg cctaaacctg gcaccagtgg
 2521 caagactgag aacgacctgg gaaaaactc ccaataaata cgaanaacta ttcaagatc
 2581 tcaagacct gttgatctc tcagaaaca tggcaaaata tctaatgtt ctcaatagtc
 2641 aaactctaca acctccata atccctctat tccagttat caaaaaggat ctacacctcc
 2701 ttacgaagg aaatgactca aaagtagacg ggctgggcaa tttgagaag ctaaggatga
 2761 ttgcaaaaga aattcgctac gttggccgaa tggcttcagt gaacatggac cctgccctca
 2821 tgttcaggac tcggaagaag aaatggcggg gttggggc tctcagccag ggtagtaca
 2881 atgcaacagt gctagatgtt gctcagacag gtgtcataa aaagcgggta cgtcgtagt
 2941 cttttctcaa tgcacaaaag ctttatgaag atgcccataa ggctcgaaaa gtgaagcagt
 3001 acctttcaa ttggagcta gaaatggacg agagagagct tcagacatta tctctcagt
 3061 gtgagccagc aaccaacaca ttgcctaaga atcctggtga caaaaagcct gtcaaatccg
 3121 agacctctcc agtagctcca agggcagggt cacaacagaa agctcagtc ctgccacagc
 3181 cccagcagca gccaccacca gcacataaaa tcaaccaggg actacaggtt cccgccgtg
 3241 cctttatcc ttacggaag aaagtgcctg taaaggatct cccacctttt ggcataaact
 3301 cctcacaagg ttaaaaaaa attctttctt tgtctgaaga aggaagtgtg gaacgtcaca
 3361 agaaacaggc tgaagataca atatacaatg catctcgca gcttctct cctctactt
 3421 ctccacagag ttctccaagg aaaggctata cttggctcc cagtgttact gtggataat
 3481 ttacagattc tggcacagt gaaattctt cacgatccag tattgttagc aattcgtct
 3541 ttgactcagt gccagctca ctgcacgatg agaggcgcca gaggcattct gtcagcatc
 3601 tggaaacaaa cctagggatg ggcaggatgg agaggcggac catgattgaa cctgatcagt
 3661 atagcttggg gtcctatgca ccaatgtccg agggccgagg cttatagct acagctacag
 3721 taattcttc tcaagcaca gaggaacttt cccaggatca gggggatcgc gcgtcacttg
 3781 atgtgtctga cagtggcctg gggagctgga cgtcatgctc aagtggctcc catgataata
 3841 tacagacgat ccagcaccag agaagctggg agactctcc attcgggcat actcacttg
 3901 attattcagg ggtatcctgca ggttatggg catcaagcag ccatatggac caaattatg
 3961 ttctgatca tagcacaag tataacaggc aaatcaaa tagagagagc ctgaacaag
 4021 cccagtcctg agcaagctgg gcgtcttcca caggttactg gggagaagac tcagaagggtg
 4081 acacaggcac aataaagcgg aggggtggaa aggatgttc cattgaagcc gaaagcagta
 4141 gcctaacgtc tgtactacg gaagaaacca agcctgtccc catgctgcc cacatagctg
 4201 tggcatcaag tactacaaag gggctcattg cacgaaagga gggcaggtat cgagagcccc
 4261 cgccacccc tcccggctac attggaattc ccattactga cttccagaa gggcactccc
 4321 atccagccag gaaaccgccc gactacaacg tggcccttca gagatcggg atggtcgcac
 4381 gatctccga cacagctggg ccttcatccg tacagcagcc acatgggcat cccaccagca
 4441 gcaggcctgt gaacaaacct cagtggcata aaccgaacga gtctgacctg cgcctcgccc
 4501 cttatcagtc ccaagggtt tccaccaggg aggatgaaga tgaacaagt tctgtgtt

(continued next page)

4561 gaggcacaga cttttctgga agcagagcga gccacctgaa aggagagcac aagaagacgt
 4621 cctgagcatt ggagccttgg aactcacatt ctgaggacgg tggaccagtt tgcctccttc
 4681 cctgccttaa aagcagcatg gggcttcttc tcccccttct ctttccctct ttgcatgtga
 4741 aatactgtga agaaattgcc ctggcacatt tcagactttg ttgcttgaaa tgcacagtgc
 4801 agcaatcttc gagctccac ttgtgctgcc tggcacatca cacagtatca ttccaaattc
 4861 caagatcatc acaacaagat gattcactct ggctgcactt ctcaatgcct ggaaggattt
 4921 ttttaactct tctttttaga tttaatcca gtcttagcac ttgatctcat tgggataatg
 4981 agaaaagcta gccattgaac tacttggggc cttaaccca ccaaggaaga caaagaaaaa
 5041 caatgaaatc ctttgagtac agtgcttgc cacttgttta caatgtcttc ctttaaaaa
 5101 aaaaaatgag tttaaagatt ttgtcagag agtaaataata tatccattta atgattacag
 5161 tatatttta aaccttaagt agggttgcca gcctgggttc tgaaaaacca aatatgccgg
 5221 acagggtgtg gccacacca gaagacggga agacctggct tgtgaccttg gcttcccatg
 5281 tcttcttgt ctcacccgag aagtgcctta tcttgggaagt atgaaatgtt agccaattaa
 5341 tacciaagaca cctcatctgc tcttcccca gtggatgggg tcttctgtga aaactgtttg
 5401 cacatggcca ggggagggaa ctaggacct tgtgtcctgt ctgagcctta tggaggcagg
 5461 acgggtgcat tggcggatgt gtctgtctcc attgagatgg atggcaaacc ccatitttaa
 5521 gtatatttc ttgatattt gtaatttag aggtgtaggt ttgtttttt gtttttgtt
 5581 ttttttaag agaaacattt ataactggat agcattgcag tgaaagcagc ttgggatgtt
 5641 ggagctaatt ccagctgttt atactgctct tcaagacag cctcccttta tgaattggc
 5701 attagggat aaacaagcct ttaaacgtga taaaagatca aaaacctggt tagacatgcc
 5761 agcctttgca aggcaggta gtcaccaaag actaacctcc aagtggcttt atggacgctg
 5821 catatagaga aggcctaagt gtagcaacca tctgtctaca gctgtatta accctataat
 5881 gactgaaatg acccctccac tctattttg tgtgttttg cacagactcc ggaaaagtga
 5941 aggtgcccaa tctgagtagt actcaaatgt gaggaactgc tggctttgga tttttttcc
 6001 attaaatca gctgatcata ttgatcagta gataaacgta aatagcttca aattttaaaa
 6061 gtggaattgc agtggttttt cactgtatca aacaatgtca gtgctttatt taataattct
 6121 ctctgtatc atggcatttg tctactgct tattacattg tcaattatgc atttgaatt
 6181 ttacatgtaa tatgcattat ttgccagttt tattatatag gctatggacc tcatgtgcat
 6241 atagaaagac agaaatctag cctaccaca agttgcaca atgttatcta agcattaagt
 6301 aatttagaa cataggactg ctaatctcag ttcgctctgt gatgtcaagt gcagaatga
 6361 caattaactg gtgatttctt catactttg atactacttg tacctgtatg tcttttagaa
 6421 agacattggt ggagtctgta tccctttgt attttaata caataattgt acatattggt
 6481 tatattttg ttgaagatgg tagaaatga ctatgttat gcttctacat ccagtttga
 6541 caagctggaa aataaataaa tataacat [SEQ ID NO: 1]

801 b.p.

1/1 31/11
 ACT AAA GGG AAC AAA AGC TGG AGC TCC ACC GCG GTG GCG GCC GCT CTA GAA CTA GTG GAT
 T K G N K S W S S T A V A A A L E L V D
 L K G T K A G A P P R W R P L * N * W I
 * R E Q K L E L H R G G G R S R T S G S

61/21 91/31
 CCC CCG GGC TGC AGG AAT TGA AGC GGT GGG AAG GAT GTC TCC GCT GAG GCA GAG AGC AGC
 P P G C R N S S G G K D V S A E A E S S
 P R A A G I Q A V G R M S P L R Q R A A
 P G L Q E F K R W E G C L R * G R E Q Q

121/41 151/51
 AGC ATG GTG CCC GTG ACT ACA GAG GAA GCC AAA CCT GTC CCT ATG CCT GCC CAC ATA GCT
 S M V P V T T E E A K P V P M P A H I A
 A W C P * L Q R K P N L S L C L P T * L
 H G A R D Y R G S Q T C P Y A C P H S C

181/61 211/71
 GTG ACG CCG AGC ACT ACC AAG GGA CTC ATC GCA CCG AAG GAA GGC AGG TAC CCG GAG CCG
 V T P S T T K G L I A R K E G R Y R E P
 * R R A L P R D S S H G R K A G T G S R
 D A E H Y Q G T H R T E G R Q V P G A A

241/81 271/91
 CCT CCC ACA CCT CCA GGC TAC GTG GGC ATC CCC ATT GCC GAT TTC CCA GAA GGG CCT TGC
 P P T P P G Y V G I P I A D F P E G P C
 L P H L Q A T W A S P L P I S Q K G L A
 S H T S R L R G H P H C R F P R R A L P

301/101 331/111
 CAC CCG GCC AGG AAG CCC CCG GAT TAC AAC GTG GCC CTG CAG CCG TCC CGC ATG GTG GCA
 H P A R K P P D Y N V A L Q R S R M V A
 T R P G S P R I T T W P C S G P A W W H
 P G Q E A P G L Q R G P A A V P H G G T

361/121 391/131
 CCG CCC ACT GAG GCC CCG GCA CCG GGC CAG ACG CCG CCT GCA GCC GCA GCC AGC CCG CCG
 R P T E A P A P G Q T P P A A A A S R P
 G P L R P R H R A R R R L Q P Q P A G R
 A H * G P G T G P D A A C S R S Q P A G

421/141 451/151
 GGC AGC AAG CCA CAG TGG CAC AAG CCC AGC GAC GCA GAC CCA CGC CTC GCG CCC TTC CAG
 G S K P Q W H K P S D A D P R L A P F Q
 A A S H S G T S P A T Q T H A S R P S S
 Q Q A T V A Q A Q R R R P T P R A L P A

481/161 511/171
 CCG CAG GCT TCG CAG GAG CCG AGG AGG ACG AAG ATG AAC AAG TGT CTG CTG TTT GAG GCG
 P Q A S Q E R R R T K M N K C L L F E A
 R R L R R S G G G R R * T S V C C L R R
 A G F A G A E E D E D E Q V S A V * G A

541/181 571/191
 CAG GCT CCT TGA TCC ACA GTG AGC CAC CCA AAG GAG AGC ACA AGA AGA CGT CCC AAG CCT
 Q A P * S T V S H P K E S T R R R P K P
 R L L D P Q * A T Q R R A Q E D V P S L
 G S L I H S E P P K G E H K K T S Q A L

601/201 631/211
 TGG AGC CTT GGC ACG CAC ATC TGA GGA TGG TGG ACC AGT TTG CCT CCT TCC CTG CCT TAA
 W S L G T H I * G W W T S L P P S L P *
 G A L A R T S E D G G P V C L L P C L K
 E P W H A H L R M V D Q F A S F P A L K

661/221 691/231
 AGC AGC ATG GGG CTT CTT CTC CCC TTC TTC CTT TCC CCT TTG CAT GTG AAA TAC TGT GAA
 S S M G L L L P F F L S P L H V K Y C E
 A - A W G F F S P S S F P L C M * N T V K
 Q H G A S S P L L P F P F A C E I L * R

(continued next page)

28/34

Figure 19B

721/241

751/251

751/251

GAA	ATT	GCC	CTG	GCA	CTT	TGC	AGA	CTT	GTT	GCT	TGA	AAT	GCA	CAG	CCC	AGC	AGC	CCC	TGA
E	I	A	L	A	L	C	R	L	V	A	*	N	A	Q	P	S	S	P	*
K	L	P	W	H	F	A	D	L	L	L	E	M	H	S	P	A	A	P	E
N	C	P	G	T	L	Q	T	C	C	L	K	C	T	A	Q	Q	P	L	S

781/261

781/261

GCT	GCT	GCC	TGC	CAC	GTC	ACG	[SEQ ID NO: 3]
A	A	A	C	H	V	T	[SEQ ID NO: 4]
L	L	P	A	T	S		[SEQ ID NO: 5]
C	C	L	P	R	H		[SEQ ID NO: 6]

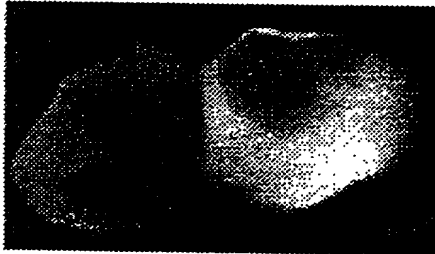
The PDZ domain (but not the C terminal SxV motif)
is required for plasma membrane localization of
GRF4

GRF4 Localization

WT



-PDZ



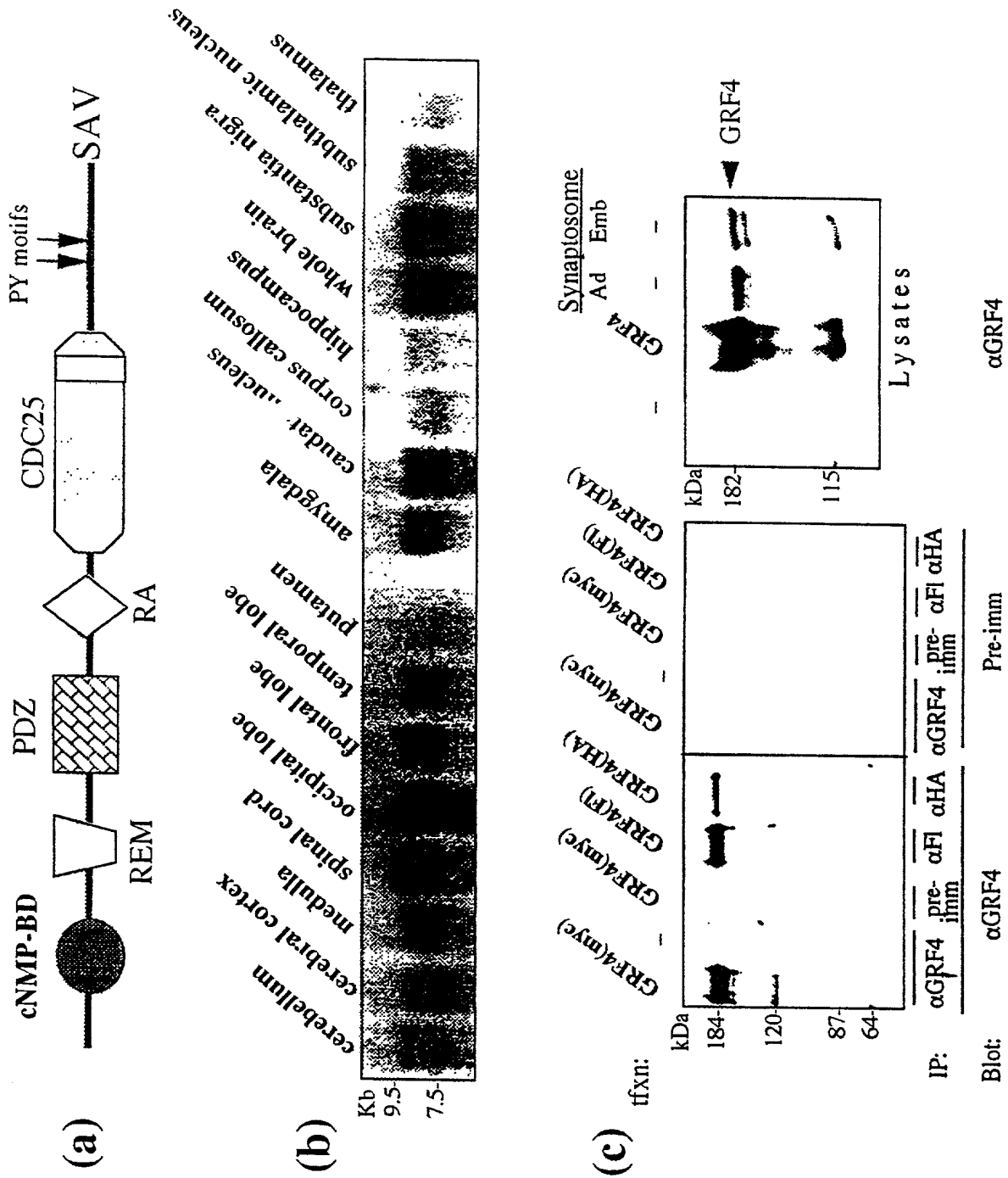
-SaV



Figure 20

FIG. 21

Figure 21



33/34



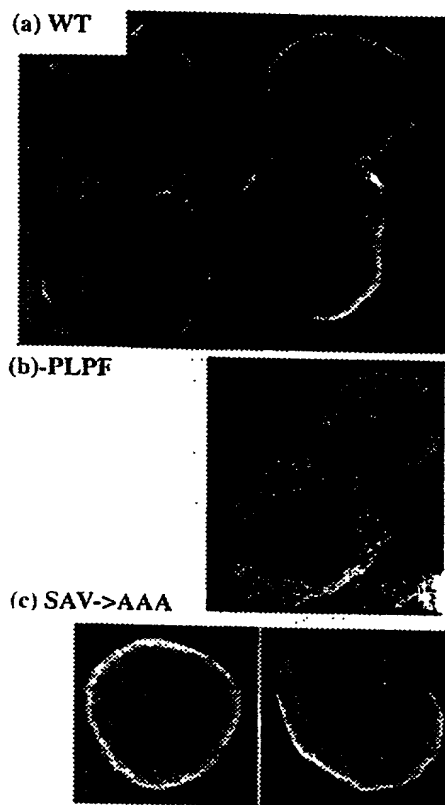


Figure 24

34/34